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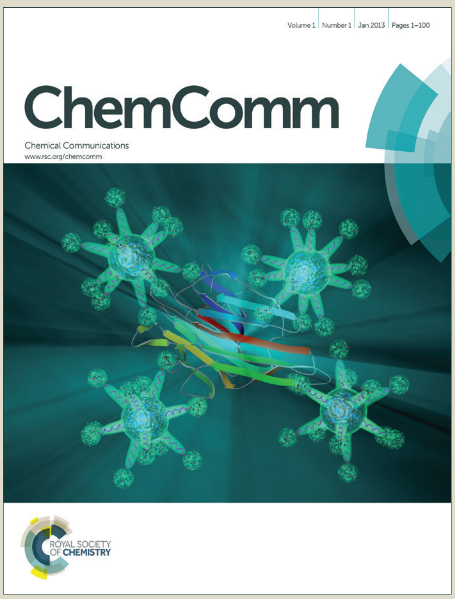

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Diastereomeric Ratio Determination by High Sensitivity Band-Selective Pure Shift NMR Spectroscopy†

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An NMR method is reported that allows diastereomeric ratios to be determined even in crowded spectra or where chemical shift differences are small compared to multiplet widths. Band-selective pure shift NMR collapses multiplets to singlets, greatly improving spectral resolution while largely retaining, or even enhancing, signal-to-noise ratio.

The intrinsic chirality of many of the building blocks of nature requires chemists to create molecules with specific stereochemical configurations, in order to govern their interactions with naturally-occurring chemical and biological species. Substrate-directable chemical reactions,[1] in which regio- and stereochemistry are controlled, are crucial for the synthesis of almost all complex natural products, and many pharmaceuticals. Measurement of the degree of stereochemical control is essential in choosing appropriate reactions and conditions, and in process optimisation. HPLC and NMR spectroscopy are used routinely to determine diastereomeric ratio (dr). However, in large molecules the presence of many overlapping signals and the small shift differences that are seen when chiral centres are well-separated can make determination of dr by NMR difficult, inaccurate, or impossible.

Much of the overlap in ^{1}H NMR spectra results from the multiplet structure caused by homonuclear scalar coupling. The recent development of 'pure shift' NMR methods allows broadband homodecoupling, collapsing multiplets to singlets and leaving a single peak for each chemically distinct site, dramatically improving spectral resolution.[2-5] However, until very recently methods for 1D pure shift ^{1}H NMR have come with a significant penalty in signal-to-noise ratio. The two most successful classes of pure shift method, based respectively on BIRD (BIlinear Rotation Decoupling)[4-7] and Zangger and Sterk's (ZS) spatially- and frequency-selective refocusing,[2, 8-11] generally cost one or two orders of magnitude in sensitivity if the whole spectrum is to be decoupled. In real-time acquisition experiments, however, it is possible to obtain simultaneous improvements in both sensitivity and resolution.[5, 7]

Here we describe the application of band-selective pure shift NMR experiments, developed in this group and the groups of Parella and Bax,[12-14] to the determination of dr. These experiments exploit - with much greater efficiency - a pulse sequence element demonstrated previously for decoupling the indirect dimension of a TOCSY experiment.[15] The real-time method enhances both sensitivity and resolution, while the interferogram-based analogue provides optimum resolution but with a modest penalty in acquisition time. Reducing overlap between reporter signals, and between reporter and other signals, allows dr to be determined more easily and more accurately, whether by integration or by line shape analysis.

Pure shift NMR methods interleave periods of free signal evolution with J-refocusing elements, that reverse the effects of couplings (scalar or dipolar[14]), while leaving those of chemical shift unchanged. The band-selective decoupling (BSD) J-refocusing element used here is simply the ZS combination of a selective and a non-selective 180° pulse, but with no gradient applied during the former. A pure shift free induction decay can be obtained either by alternating periods of free evolution and J-refocusing during data acquisition (real-time methods), or by constructing an equivalent decay from short chunks of free induction decay measured using a single evolution time t_1 with a J-refocusing element at its centre (interferogram methods). In each case the result of Fourier transformation is an NMR spectrum with a singlet at each chemical shift. The advantage of BSD methods is that all the available spins contribute to the required signal, rather than the small minority seen in BIRD and ZS experiments.

In the determination of dr the chemist is generally interested only in the integral ratios of a few diagnostic signals, typically those of protons lying close to the stereogenic centres of interest; the rest of the spectrum is superfluous. It is therefore much more efficient to

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acquire a BSD pure shift NMR spectrum, containing only the spectral region of interest with couplings to spins outside the region suppressed, rather than measuring a complete pure shift spectrum at a large penalty in the measurement time required.

Conventional selective (or band-selective) homonuclear decoupling is performed by irradiating a single ^{1}H multiplet during acquisition using coherent irradiation[16] (or a shaped RF pulse[17]) in time-shared mode.[18, 19] Band-selective time-shared experiments have to target all the spins to which the spins of interest are coupled, are particularly challenging to set up because of Bloch-Siegert and off-resonance effects, and are relatively little used. Pure shift BSD experiments, in contrast, target only the spins of interest and are much simpler.

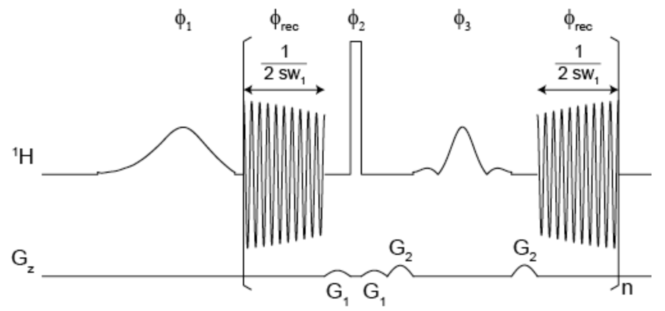

Figure 1: Real time pure shift sequence using a 90° selective excitation pulse (eBURP) with phase φ_1, and a J-refocusing element comprising a broadband 180° pulse with phase φ_2 and a band-selective (rSNOB) 180° refocusing pulse with phase φ_3. Data points are collected using a looped, windowed acquisition with phase φ_{rec}. The J-refocusing and acquisition loop is repeated ***n*** times until the required number of data points have been collected. Phase cycle: ϕ_1 = *x,-x,-x,x,y,-y,-y,y*; ϕ_2 = *x,-x*; ϕ_3 = *-x,x*; ϕ_{rec}= ϕ_1. The duration 1/sw$_1$ of the acquisition chunks depends on the balance required between resolution and spectral purity; a good compromise is an sw$_1$ roughly twice the maximum multiplet width in the normal spectrum.

The real-time BSD pure shift pulse sequence used, which has at its core an analogue of the most commonly-used variant of the ZS pulse sequence,[8, 10] is shown in Figure 1. The magnetic field gradient usually applied during the ZS selective refocusing pulse is removed, to give selective refocusing of a defined band of chemical shifts. Equivalent methods have been described recently for band-selective pure shift NMR of peptides[13, 14]. The 180° refocusing pulse is tailored to affect a band of chemical shifts containing only one multiplet from each spin system, so that all couplings are refocused and only singlets result in the final spectrum.

The real-time sequence shown refocuses the effects of J between short chunks of data acquisition. This prevents the effects of J-evolution from building up, so only the effects of the chemical shift are observed in the FID and resultant spectrum.[5, 7, 10] In the analogous interferogram sequence (See ESI, Figure S1), each chunk of data is acquired in a separate experiment as a chemical shift evolution time t_1 is incremented, just as in a two-dimensional NMR experiment.[4, 8] The J-evolution is prefocused so that at the centre of each chunk only chemical shift terms have accumulated. The real-time method of Figure 1 requires approximately the same experiment time as a conventional measurement, and offers similar or better sensitivity. However, relaxation during the successive selective pulses does slightly reduce the resolution advantage, which is not the case for the interferogram method. The disparity depends on the bandwidth; the wider the region of interest, the shorter the selective pulses and the smaller the extra relaxation contribution. Thus while the real-time experiment is preferable for most purposes, the interferogram experiment comes into its own where the region of interest is narrow and where resolution is paramount. In both experiments relaxation during the pulse sequence affects the signal integrals measured slightly, but such effects would only become a problem in systems with relaxation too fast for pure shift methods to offer much resolution advantage.

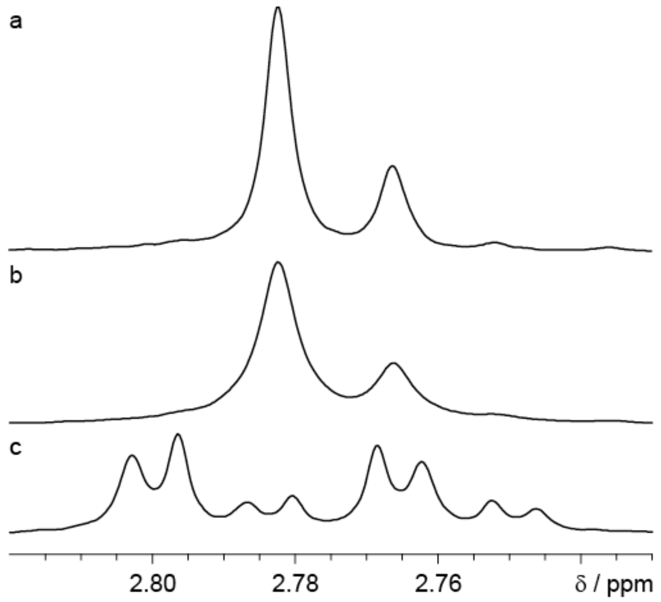

Figure 2: 500 MHz ^{1}H NMR spectra showing dr reporter signals for a mixture of hesperidin diastereomers in d$_6$-DMSO acquired using a) interferogram-based and b) real-time band-selective pure shift experiments, and c) standard acquisition. Experimental and processing parameters were consistent between the two experiments; full details are given in the ESI.

Where signal resolution remains a problem even after the use of pure shift methods, a chemical additive may be introduced that can separate signals. The traditional additive would be a chiral lanthanide shift reagent[20] but other chiral species can also provide good resolution of diastereomer,[21] and even enantiomer, signals. Conversely, if NMR multiplet signals are not fully resolved after addition of additives the subsequent use of band-selective pure shift NMR may allow their separation and quantification.[22]

The benefits of band-selective pure shift NMR for quantifying dr are illustrated in Figure 2 for the flavanone glycoside hesperidin. Hesperidin exists naturally as a mixture of two diastereomers (see ESI, Figure S2) in many citrus fruits. The dr is dependent on the species of citrus, the ripeness of the fruit, and the temperature at which extraction occurred.[23] Two sets of signals, one from each diastereomer, occur in the ^{1}H NMR spectrum, at the chemical shifts shown in Figure 2. The signals in the normal spectrum (Figure 2c) are

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split into overlapping doublets of doublets, making quantification of dr difficult. Using the band-selective pure shift method of Figure 1 collapses the multiplets, making integration and hence determination of dr straightforward.

In a second example, the crude product mixture of a remotely diastereoselective reaction entailing foldamer-mediated 1,61 stereocontrol[12] contained two diastereomers (epimeric at the benzylic chiral centre, see ESI, Figure S3) and several minor impurities. The diastereoselectivity of the reaction, in which facially selective attack on an acyliminium ion was induced through an extended achiral helix, could not be determined confidently by standard ^{1}H NMR methods because of signal overlap. Changing solvent from d_4-MeOD to d_8-THF allowed the two diastereomer signals to be distinguished, only to reveal underlying impurity signals (Figure 3b). The combination of low available concentration and a single reporter region made the band-selective pure shift method particularly attractive here. Even at 800 MHz, using the higher resolution interferogram-based experiment (See ESI, Figure S1), a shoulder is visible on the main diastereomer signal. Lorentzian line shape fitting rather than direct integration was therefore used, giving a dr of 12:88 rather than drs of 8:92 and 7:93 obtained by integration of the pure shift and conventional ^{1}H spectra respectively. The dr obtained by lineshape fitting of the pure shift NMR data was consistent with the expected degree of local asymmetry induced by the achiral helix mediating the remote asymmetric induction.

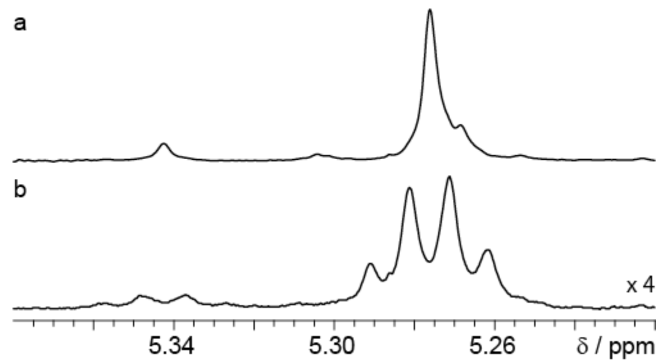

Figure 3: 800 MHz ^{1}H NMR spectra of the enamide 1,61-asymmetric induction product of an oligomeric peptide dissolved in d_8-THF (see ESI and ref. 12). The multiplet structure in the standard proton experiment (b) is collapsed in the interferogram-based pure shift NMR experiment (a) where signal intensity also increases. Experimental and processing parameters are consistent for the spectra and are given in the ESI.

Conclusions

NMR is a valuable tool for measurement of diastereomeric ratios, whether of diastereomeric materials or of diastereomers made from chiral substrates using chiral derivatising or chemical shift reagents, but resolution and sensitivity are critical. Band-selective pure shift NMR methods both simplify the determination of diastereomeric ratio and improve its reliability.

Notes and references

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